



# STIC Search Report

## Biotech-Chem Library

STIC Database Tracking Number: 123456

**TO:** Leon Y Lum  
**Location:** REM/3D78/3C70  
**Art Unit:** 1641  
**Sunday, August 15, 2004**  
**Case Serial Number:** 10/044708

**From:** Paul Schulwitz  
**Location:** Biotech-Chem Library  
REM-1A65  
**Phone:** (571)272-2527

**paul.schulwitz@uspto.gov**

### Search Notes

Examiner Lum,

See attached results.

If you have any questions about this search feel free to contact me at any time.

Thank you for using STIC search services!

Paul Schulwitz  
Technical Information Specialist  
STIC Biotech/Chem Library  
(571)272-2527

## SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Leon Lum Examiner #: 80278 Date: 8/9/04  
 Art Unit: 1641 Phone Number 302-2878 Serial Number: 10/044708  
 Mail Box and Bldg/Room Location: Rosenzweig Bldg. Results Format Preferred (circle): PAPER DISK E-MAIL  
Mailbox: 3C70  
Room: 3D78

If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc. if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Isotope-coded ionization-enhancing reagents (ICIER) for high-throughput protein identification and quantitation using matrix-assisted laser desorption ionization mass spectrometry  
 Inventors (please provide full names): Yong-dong Qiu, Jack Wang, Rodney M. Henrick, Jack H. Wang

Earliest Priority Filing Date: 10/23/2000

\*For Sequence Searches Only\* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

Please search the structures of Claim 9 (see attached) and the  
 following search terms:  
 $\Delta = \text{Centenium}$

Matrix Assisted Laser Desorption / Ionization (MALDI)

Mass Spectrometry (MS)

MALDI-MS

Isotope Coded Affinity Tag (ICAT)

STAFF USE ONLY		Type of Search	Vendors and cost where applicable
Searcher:		NA Sequence (#)	STN <u>730-92</u>
Searcher Phone #:		AA Sequence (#)	Dialog
Searcher Location:		Structure (#)	<u>2</u> Questel/Oribit
Date Searcher Picked Up:	<u>8/5</u>	Bibliographic	Dr. Link
Date Compiled:		Litigation	Lexis/Nexis
Searcher Prep & Review Time:	<u>20</u>	Fulltext	Sequence Systems
Clerical Prep Time:	<u>100</u>	Patent Family	WWW/Internet
Online Time:	<u>25</u>	Other	Other (specify)

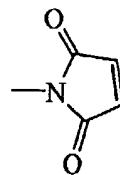
WHAT IS CLAIMED IS:

1. A method for enhancing identification and relative quantitation of proteins and peptides using mass spectrometry (MS), said method comprising the steps of:

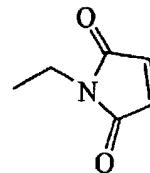
- (a) reducing the disulfide bonds of a first sample from a biological mixture containing proteins and peptides;
- (b) labeling proteins and peptides in the first sample with a reagent which comprises a thiol-specific reactive group attached to a guanadino group via a linker which can be differentially labeled;
- (c) separating the proteins and peptides from the sample;
- (d) digesting the proteins to provide a mixture containing digestion peptides and peptides from the first sample; and
- (e) subjecting the peptides of (d) to quantitative MS analysis and protein identification.

2. The method according to claim 1, wherein the peptides of (d) are subjected to matrix-assisted laser desorption/ionization (MALDI) - MS.

3. The method according to claim 1, wherein the reagent comprises a thiol-specific reactive group selected from the group consisting of  $\alpha$ -haloacetyl (-X-CH<sub>2</sub>CO-, X = I, Br, or Cl) or a maleimide group having a structure selected from the group consisting of:



and



12,29 - 12,54

(25)

4. The method according to claim 1, wherein the linker comprises an alkyl chain having three to eight carbon atoms, optionally substituted with one or more amido groups, carboxy groups, or amino groups.

5. The method according to claim 1, wherein the proteins and peptides are further subjected to peptide mass mapping, said method further comprising the steps of:

labeling proteins and peptides in a second sample with said reagent having heavy stable isotopes; and

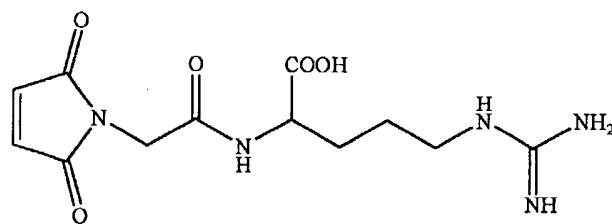
mixing the first and second samples prior to the separation step,  
wherein the reagent in the labeling step contains light stable isotopes.

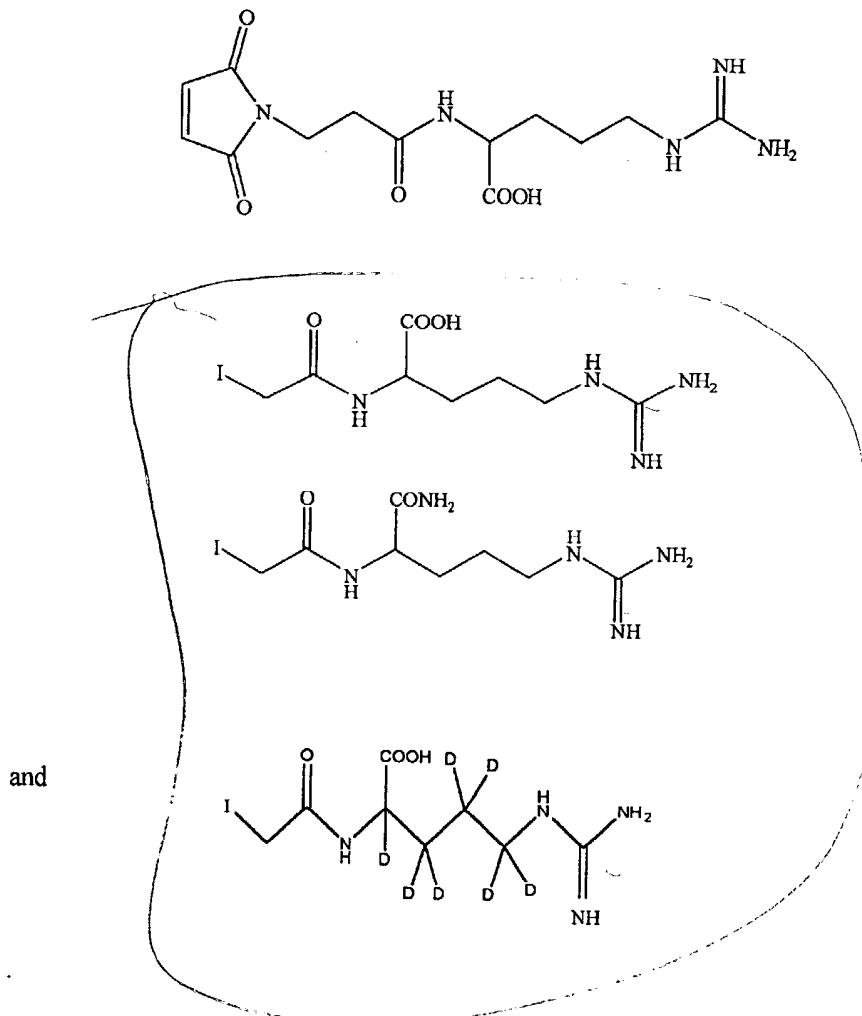
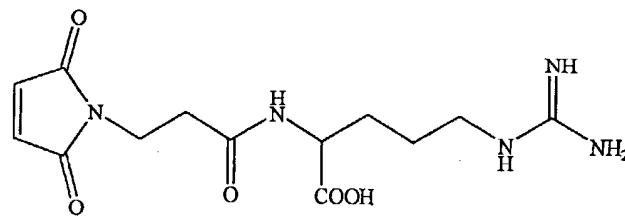
6. The method according to claim 1, wherein the linker in the reagent of step (b) contains a substitution of four to twelve atoms with a stable isotope.

7. The method according to claim 6, wherein the linker contains seven stable isotopes.

8. The method according to claim 6, wherein the hydrogen atoms are substituted with deuterium.

9. The method according to claim 5, wherein the reagent is selected from the group consisting of:





10. The method according to claim 5, wherein the separation step is performed using one dimensional or two dimensional polyacrylamide gel electrophoresis (1D or 2D-PAGE), or liquid chromatography.

11. The method according to claim 1, wherein the digestion step is performed in-gel or in solution.

12. A method for preparing peptides for MALDI-MS and subsequent data analysis, said method comprising the steps of:

(a) reducing the disulfide bonds of proteins from biological samples;

(b) labeling proteins in one sample with a reagent which comprises a thiol-specific reactive group attached to a guanidino group via a linker which is differentially labeled with light stable isotopes;

(c) labeling proteins in a second sample with a reagent having heavy stable isotopes;

(d) mixing the first and second labeled samples;

(e) separating the proteins from the mixture;

(f) digesting the proteins, thereby providing peptides ready for MALDI-MS analysis and protein identification.

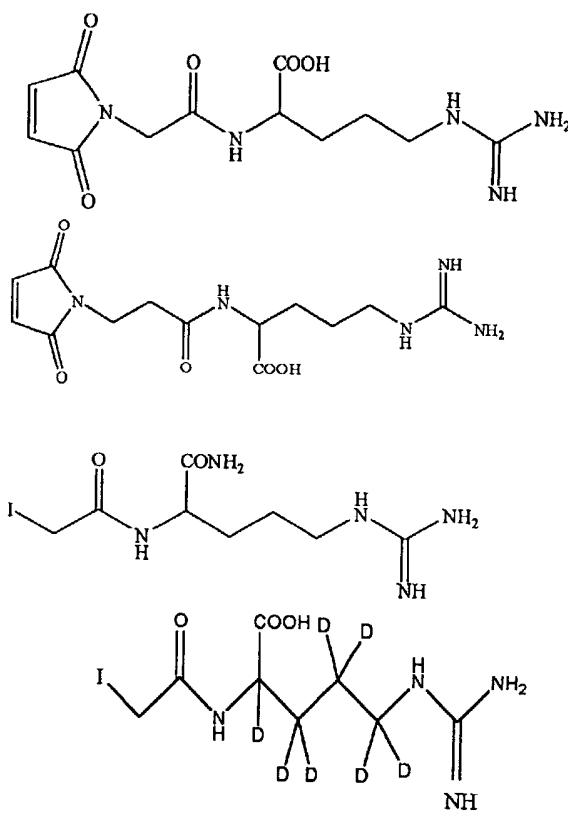
13. The method according to claim 11, wherein the digestion step is performed using trypsin.

14. A compound useful in quantitative analysis of protein mixtures, said compound comprising a thiol-specific reactive group attached to a guanidino group via a linker which can be differentially labeled with stable isotopes.

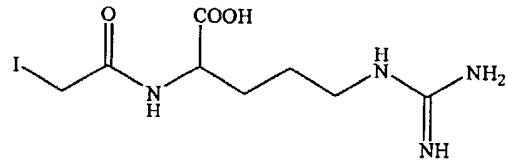
15. The compound according to claim 14, wherein the linker contains four to twelve stable isotopes.

16. The compound according to claim 14, wherein the linker contains a substitution of at least six hydrogen atoms with deuterium.

17. The compound according to claim 14, selected from the group consisting of:



and

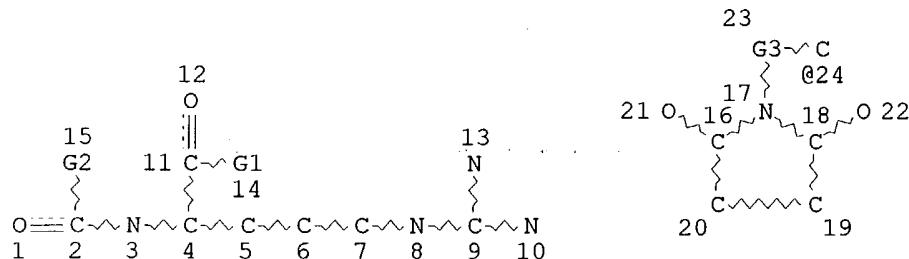


18. A reagent kit for the analysis of proteins by mass spectrometric analysis that comprises a compound of claim 14 or claim 17.

19. The reagent kit according to claim 18, comprising a set of substantially identical differentially labeled alkylating reagents.

20. The reagent kit according to claim 18, further comprising one or more proteolytic enzymes for use in digestion of proteins modified by said compounds.

=> d que  
L13 STR



I ~ C  
25 @26

```
VAR G1=OH/NH2
VAR G2=24/26
REP G3=(0-4) C
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED
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GRAPH ATTRIBUTES:  
RING(S) ARE ISOLATED OR EMBEDDED  
NUMBER OF NODES IS 26

STEREO ATTRIBUTES: NONE

L15 2 SEA FILE=REGISTRY SSS FUL L13  
L16 1 SEA FILE=HCAPLUS ABB=ON PLU=ON L15

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L16 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2004 ACS on STN  
ACCESSION NUMBER: 1968:78612 HCAPLUS  
DOCUMENT NUMBER: 68:78612  
TITLE: Potential antiviral agents. Carbobenzoxy di- and tripeptides active against measles and herpes viruses  
AUTHOR(S): Nicolaides, Ernest D.; De Wald, Horace A.; Westland, Roger D.; Lipnik, Marilyn; Posler, Jeanette  
CORPORATE SOURCE: Parke, Davis and Co., Ann Arbor, MI, USA

SOURCE: Journal of Medicinal Chemistry (1967), 11(1), 74-9  
 CODEN: JMCMAR; ISSN: 0022-2623

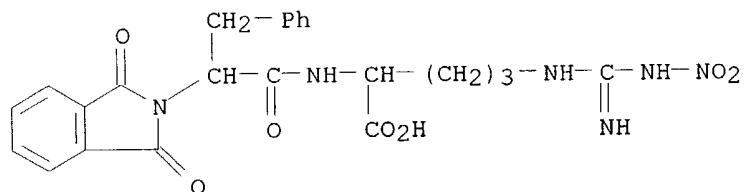
DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB A large number of carbobenzoxy dipeptides, several tripeptides, and a number of alkyl, cycloalkyl, aryl, and heterocyclic amide derivs. of carbobenzoxy-L-and D-phenylalanine were synthesized. Many of the peptides were active against measles and herpes viruses.

IT 17461-57-3P  
 RL: SPN (Synthetic preparation); PREP (Preparation)  
 (preparation of)

RN 17461-57-3 HCAPLUS

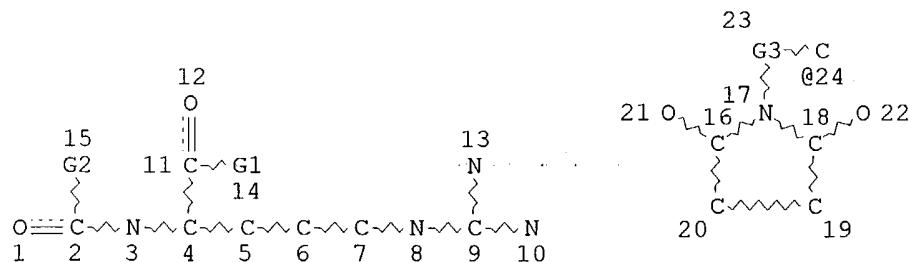
CN Ornithine, N5-(nitroamidino)-N2-(L- $\alpha$ -phthalimidohydrocinnamoyl)-, L-  
 (8CI) (CA INDEX NAME)



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L13

STR



I~C  
25 @26

VAR G1=OH/NH2  
 VAR G2=24/26  
 REP G3=(0-4) C  
 NODE ATTRIBUTES:  
 DEFAULT MLEVEL IS ATOM  
 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:  
 RING(S) ARE ISOLATED OR EMBEDDED  
 NUMBER OF NODES IS 26

STEREO ATTRIBUTES: NONE  
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 L20 1 SEA FILE=MARPAT ABB=ON PLU=ON L19/COM

=> d l20 ibib abs qhit

L20 ANSWER 1 OF 1 MARPAT COPYRIGHT 2004 ACS on STN  
 ACCESSION NUMBER: 128:34581 MARPAT  
 TITLE: Preparation of acetylene derivatives for inhibition of matrix metalloproteases  
 INVENTOR(S): Dixon, Brian R.; Chen, Jinshan  
 PATENT ASSIGNEE(S): Bayer Corporation, USA; Dixon, Brian R.; Chen, Jinshan  
 SOURCE: PCT Int. Appl., 71 pp.  
 CODEN: PIXXD2

DOCUMENT TYPE:

Patent

LANGUAGE:

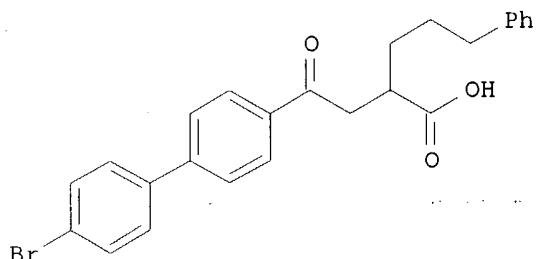
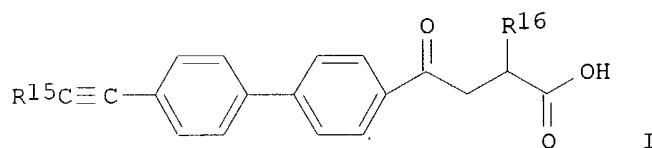
English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743245	A1	19971120	WO 1997-US7921	19970512
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
ZA 9704031	A	19980219	ZA 1997-4031	19970509
HR 970245	B1	20020630	HR 1997-970245	19970509
AU 9729386	A1	19971205	AU 1997-29386	19970512
AU 710759	B2	19990930		
EP 912496	A1	19990506	EP 1997-923622	19970512
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
BR 9709077	A	19990803	BR 1997-9077	19970512
CN 1225623	A	19990811	CN 1997-196456	19970512
JP 11511179	T2	19990928	JP 1997-540980	19970512
JP 3090957	B2	20000925		
TW 381079	B	20000201	TW 1997-86106283	19970512
PRIORITY APPLN. INFO.:			US 1996-645028	19960515
			WO 1997-US7921	19970512

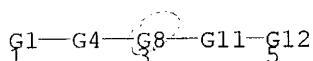
GI



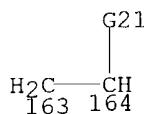
AB The title compds. [I; R15 = HOCH<sub>2</sub>, MeOCH<sub>2</sub>, CH<sub>3</sub>CO<sub>2</sub>CH<sub>2</sub>, EtOCO<sub>2</sub>CH<sub>2</sub>, HO(CH<sub>2</sub>)<sub>2</sub>, CH<sub>3</sub>CO<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>, HO<sub>2</sub>C(CH<sub>2</sub>)<sub>2</sub>, OHC(CH<sub>2</sub>)<sub>3</sub>, HO(CH<sub>2</sub>)<sub>4</sub>, Ph, etc.; R16 = Ph(CH<sub>2</sub>)<sub>3</sub>, phthalimidoethyl] are prepared I are useful for inhibiting matrix

metalloproteases and, therefore, combating conditions to which MMP's contribute, such as osteoarthritis, rheumatoid arthritis, septic arthritis, periodontal disease, corneal ulceration, proteinuria, aneurysmal aortic disease, dystrophic epidermolysis, bullous, conditions leading to inflammatory responses, osteopenias mediated by MMP activity, temporomandibular joint disease, demyelinating diseases of the nervous system, tumor metastasis or degenerative cartilage loss following traumatic joint injury, and coronary thrombosis from atherosclerotic plate rupture. Thus, compound (II) was reacted with HOCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>CO<sub>2</sub>H in the presence of Et<sub>2</sub>NH, CuI, and trans-dichlorobis(triphenylphosphine)palladate to give I [R<sub>15</sub> = HOCH<sub>2</sub>, R<sub>16</sub> = Ph(CH<sub>2</sub>)<sub>3</sub>], which showed IC<sub>50</sub> of 21 μM against MMP-3.

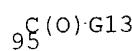
## MSTR 2



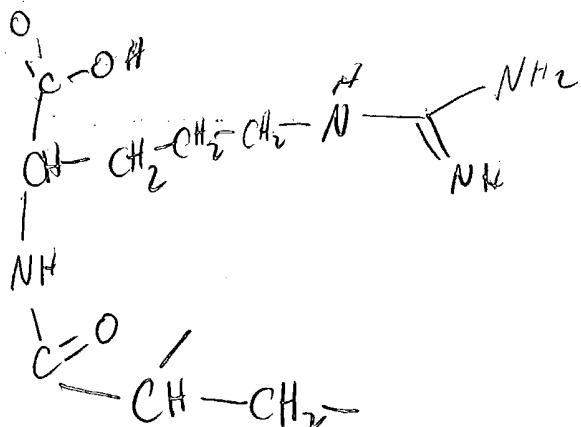
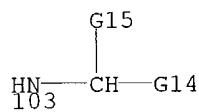
$$\text{G11} = 163-3 \ 164-5$$



$$\text{G12} = 95$$



$$\text{G13} = 103$$



$$\text{G14} = \text{CO}_2\text{H}$$

$$\text{G15} = \text{CH}_2\text{CH}_2\text{CH}_2\text{NHC(NH)}\text{NH}_2$$

$$\text{G22} = (1-5) \text{ CH}_2$$

$$\text{G24} = \text{phthalimido}$$

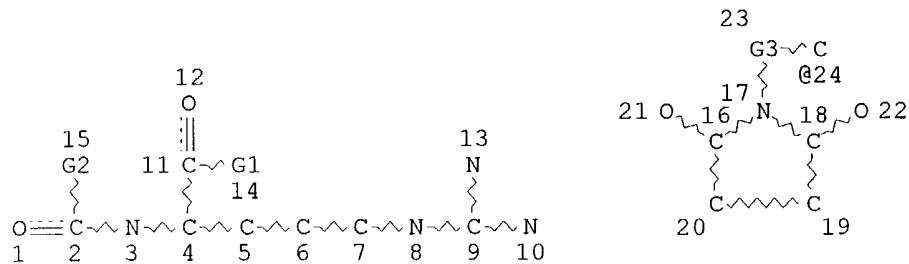
DER: and pharmaceutically acceptable salts

MPL: disclosure

NTE: Ak in G11 may contain cyclic groups

=> d que  
L13

STR



I ~ C  
25 @26

```
VAR G1=OH/NH2  
VAR G2=24/26  
REP G3=(0-4) C  
NODE ATTRIBUTES:  
DEFAULT MLEVEL IS ATOM  
DEFAULT ECLEVEL IS LIMITED
```

GRAPH ATTRIBUTES:  
RING(S) ARE ISOLATED OR EMBEDDED  
NUMBER OF NODES IS 26

STEREO ATTRIBUTES: NONE  
L17 0 SEA FILE=BEILSTEIN SSS FUL L13